

Functional coupling of acoustic and chemical signals in the courtship behaviour of the male *Drosophila melanogaster*

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During courtship, the male *Drosophila melanogaster* sends signals to the female through two major sensory channels: chemical and acoustic. These signals are involved in the stimulation of the female to accept copulation. In order to determine the respective importance in the courtship of these signals, their production was controlled using genetical and surgical techniques. Males deprived of the ability to emit both signals are unable to mate, demonstrating that other (e.g. visual or tactile) signals are not sufficient to stimulate the female. If either acoustic or chemical signals are lacking, the courtship success is strongly reduced, the lack of the former having significantly more drastic effects. However, the accelerated matings of males observed with males bearing wild-type hydrocarbons compared with defective ones, whichever the modality of acoustic performance (wing vibration or playback), strongly support the role of cuticular compounds to stimulate females. We can conclude that among the possible factors involved in communication during courtship, acoustic and chemical signals may act in a synergistic way and not separately in *D. melanogaster*.

Keywords: courtship; acoustic communication; chemical communication; mutant; *Drosophila*

1. INTRODUCTION

Courtship is usually considered to have two major functions: species recognition and sexual stimulation of the mate. The fruitfly *Drosophila melanogaster*, is a particularly suitable model for studying signals involved in the success of courtship. In this Dipteran species, the courtship consists of sequential stereotyped elements of behaviour that are primarily under genetic control (Hall 1994; Yamamoto *et al.* 1997). Courtship sequences have been extensively studied (e.g. Bastock & Manning 1955; Manning 1959; Cowling & Burnet 1981; Cobb *et al.* 1986; Welbergen *et al.* 1987). They occur in a fixed order, beginning with a male orientating towards the female and then following her. The male taps the cuticle of the female with his foretarsi, spreads one or two wings and vibrates, producing a courtship song. The male then licks the genitalia of the female with his proboscis and attempts to copulate (Hall 1994; Greenspan 1995; O'Dell & Kaiser 1997).

For a copulation attempt to be successful, the female has to be receptive and ready to mate. So the male has to appeal to the female by means of at least four kinds of signals: visual, chemical, tactile and acoustic (Ewing 1983). Such signals carry information about species, gender and receptivity, and are used to modulate the responses of either the male or the female. Fruitflies are sexually dimorphic for cuticular hydrocarbons (Antony *et al.* 1985; Jallon & David 1987) and it has been suggested that specific hydrocarbons produced by the male on his cuticle have a critical role in stimulating the female (Tompkins *et al.* 1982; Gailey *et al.* 1986), especially the compound 7-tricosene (Jallon 1984; Ferveur & Jallon

1993; Scott 1994). Acoustic signals produced by the male towards the female are also important during courtship: the mating success of males was reduced when their wings were cut, that is, when they were mute (Sturtevant 1915). The courtship song produced by the male exhibits two kinds of signals, a pulse song (intermittent series of rapid clicks) and a sine song (pure sinusoidal continuous hums), both of which play a critical role in species recognition (Bennet-Clark & Ewing 1969; Kyriacou & Hall 1982; Ritchie *et al.* 1999) and female stimulation (Schilcher 1976; Kyriacou & Hall 1984).

Our aim was to investigate the relative influence of acoustic and chemical signals emitted by the male on courtship success. Effectively, as seen above, the importance of acoustic and chemical signals have been intensively but separately studied. To our knowledge, however, no studies have focused on how both signals could act synergistically on courtship success. We studied these courtship signals using genetical or surgical techniques that allowed control of the production of only one or the other type of signal. To drastically reduce the production of cuticular hydrocarbons we used genetically modified males, that is males whose *transformer* gene (*tra*) was manipulated. To stop the production of acoustic signals, we surgically removed the wings to obtain mute males. In a first step we compared the courtship success and the courtship song production of winged males with or without normal cuticular profiles, in order to verify that the lack of cuticular hydrocarbons does not lead to an abnormal courtship behaviour. In a second step we compared the courtship success of males in the presence of virgin females in four experimental situations: mute males with an abnormal cuticular profile, mute males with a normal cuticular profile, mute males with an abnormal cuticular profile and courtship song played back and mute males

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with a normal cuticular profile and courtship song played back.

2. MATERIAL AND METHODS

(a) *Flies*

Females were from the Canton S (CS) strain of *D. melanogaster*. Males resulted from a cross between homozygous *UAS-tra* females, carrying the *UAS-tra* feminizing transgene (Ferveur *et al.* 1995; Ferveur & Sureau 1996), and homozygous P[GAL4-Hsp70.PB] males (Brand *et al.* 1994). The *hsp70-GAL4* transgene makes it possible to produce the GAL4 protein ubiquitously after heat shock, which in turn activates the *UAS-tra* transgene (Ferveur *et al.* 1997; Savarit *et al.* 1999). The over-expression of *tra* under the control of the heat-shock Hsp 70 promoter (Brand & Perrimon 1993) occurs ubiquitously at early imaginal life and leads to a significant reduction of cuticular hydrocarbons (Savarit 1999; Ferveur & Savarit 2000). Six-hour old males were transferred from vials to polypropylene tubes (one male per tube) in a 37 °C water bath for 1 h. After this heat shock these males, termed *hs-tra*, were put again into vials containing fresh medium and housed alone until they reached 4 days of age. Males not subjected to heat shock, termed *nhs-tra*, were otherwise treated in exactly the same way. For control purposes we also used males resulting from a cross between homozygous *UAS-lacZ* females and homozygous P[GAL4-Hsp70.PB] males. These males, termed *hs-lacZ*, were subjected to the same heat-shock conditions as above.

(b) *Rearing conditions*

Flies were raised on a cornmeal/agar/molasses/yeast medium, in controlled temperature and light conditions at 25 °C with a 12 L:12 D cycle and lights on at 09.00. Virgin males and females were sexed within 1 h of eclosion under light CO₂ anaesthesia. Females were placed in groups while males were placed alone into vials with fresh medium.

When required, wings were removed from males at the time of sexing using fine scissors and forceps: the wing was spread from the thorax using the forceps and then cut at the level of the humeral cross vein (see the 'A' cut in Ewing (1964)). According to Ewing, this A cut removed at least 90% of the wing area and leads to a reduction of the percentage of copulations by over 80%. We were not able to detect any acoustic signals produced by males that had been operated on, in a control recording experiment. Flies were 4 days old when tested.

(c) *Hydrocarbon analysis*

Each male was placed alone in a micro vial containing 50 µl of hexane, and left there for 10 min. Each was then rapidly removed from the solvent and 20 µl of internal standard containing a constant amount of hexacosane (800 ng) was added. This procedure follows that described by Jallon *et al.* (1988) and modified by Ferveur (1991). Cuticular hydrocarbons were analysed by gas chromatography. The absolute quantities and the relative levels (as percentages of total amount) of all detectable hydrocarbons were calculated for each male. Identification of peaks was carried out using a GCMS mass spectrometer MD800 (Thermoquest) coupled with an 8060 Fisons Gas Chromatograph equipped with a 25 m column. The GCMS was equipped with MASSLAB 1.2.7 software.

(d) *Courtship song analysis*

(i) *Recording method*

To record the signals emitted by the *hs-tra* and *nhs-tra* males we used the method described in detail by Aubin *et al.* (2000). Briefly, this method is based upon the following principle: two simultaneous recordings were made using two microphones at a distance of 6 cm apart. One microphone recorded the acoustic signals produced by the flies placed on the microphone in a chamber plus the ambient noise while the second microphone recorded only the ambient noise. Then a simple subtraction between these two recordings allowed for the isolation of the courtship song, with a good signal-to-noise ratio.

The microphones used were two 4176 Bruel & Kjaer half inch prepolarized condenser microphones. The output of the microphones were flat within 2 dB between 6.5 Hz and 12.5 kHz. The signals were recorded simultaneously from both microphones on the two channels of a Sony TCD 3 stereo DAT recorder (frequency response curve flat within 1 dB between 20 Hz and 20 kHz) at a sampling frequency of 48 kHz for each channel. Signal-to-noise ratio and dynamic range were both more than 90 dB. The signals recorded on the two channels of the magnetic tape of the DAT corresponded to 16 bits of digital data. These data were transferred to a PC computer by means of an Audiomedia III PCI acquisition card interface and then stored as files (one for each channel) on the hard disk of the computer for all subsequent analysis. With this method, there was no time delay between the data in both files. Then a subtraction between files was made, using a program written in C language, thus isolating the signals produced by the flies. This signal was examined on a computer generated oscillographic representation for temporal analysis. Temporal measurements were made directly on the computer screen with the help of a cursor with a reference time base, and automatically stored in ASCII files for subsequent analysis. Time resolution as measured on the screen was 125×10^{-6} s.

(ii) *Parameters measured*

The courtship songs of 10 males *hs-tra* and 10 males *nhs-tra* were recorded and analysed. During courtship, males emit two categories of signals: the pulse song and the sine song. The pulse song is composed of short sequences (bursts) of sound units (pulses) separated by a specific interpulse interval (IPI). The sine song is composed of sinusoidal hums (termed bouts).

The following parameters were quantified: IPI, burst duration (BD), number of pulses per burst (NPB) and percentage of courtship spent in pulse song emission (%PS) for the pulse song, bout of sine song duration (BSSD) and percentage of courtship spent in sine song emission (%SS) for the sine song.

(e) *Mating chambers*

We used mating chambers (7 mm inside diameter \times 3 mm high) with transparent nylon net floors. The chambers were included in a plexiglas floor wheel placed on a nylon mesh. A roof wheel, with a small hole, was superimposed on a floor wheel, which could rotate on a central axis. Flies were introduced through the hole.

(f) *Playback experiment design*

Experimental signals were played back using a Sony TCD 3 DAT recorder and transmitted to a Soshin loudspeaker (50 mm diameter, 8 Ω) via a 10 W power amplifier built in the laboratory (frequency response: 100–5600 Hz \pm 2 dB). Experimental signals were played at a mean sound pressure level (SPL) of 80 dB,

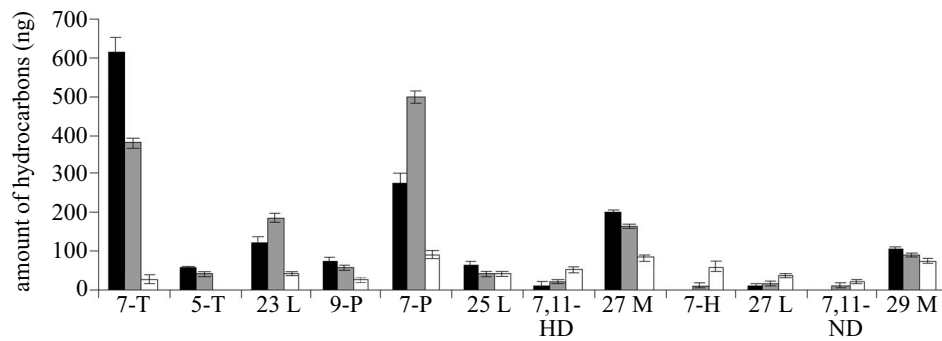


Figure 1. Cuticular hydrocarbons produced by 4 days Hsp70-GAL4 \times UAS-*tra* and Hsp70-GAL4 \times UAS-*lacZ* *Drosophila melanogaster* males subjected (hs-*tra* and hs-*lacZ*) or not (nhs-*tra*) to a heat-shock in early imaginal life (mean \pm s.e.) (Black bars, hs-*lacZ* males ($n = 10$); grey bars, nhs-*tra* males ($n = 54$); white bars, hs-*tra* males ($n = 57$)). Abbreviations: 7-T, 7-tricosene; 5-T, 5-tricosene; 23 L, *n*-tricosane; 9-P, 9-pentacosene; 7-P, 7-pentacosene; 25 L, *n*-pentacosane; 7,11-HD, 7,11-heptacosadiene; 27 M, 2-methyl-hexacosane; 7-H, 7-heptacosene; 27 L, *n*-heptacosane; 7,11-ND, 7,11-nonacosadiene; 29 M, 2-methyl-octacosane. These hydrocarbons shown on histograms represent more than 94% of the total hydrocarbons detected. The difference between nhs-*tra* and hs-*tra* is statistically significant for each compound, except for the 7,11-ND.

and measured with a 4176 Bruel & Kjaer prepolarized condenser microphone at the level of the floor of the observation cell. The mating chambers were placed on a nylon mesh above the loudspeaker.

From a previous courtship song analysis (Aubin *et al.* 2000) we selected one natural song, including both pulse and sine songs that was representative of the species. It was produced by a mature male *D. melanogaster* during a successful courtship towards a mature virgin female. The duration of the signal played back was 30 min. Most other studies in this area relied on artificial song playbacks. We have chosen a natural complete song because it carried both natural frequencies and a natural pattern of emission (and presumably the natural periodicity of IPI production discovered by Kyriacou & Hall (1980)).

(g) Experimental conditions

Two separate sets of experiments were performed with a single male in the presence of a virgin CS female. In the first experiment the courtship success and courtship song of winged nhs-*tra* and hs-*tra* males were compared. In the second experiment courtships of wingless (mute) nhs-*tra* and hs-*tra* males were compared, with courtship songs played back or not. The use of wingless males with songs played back allowed us to control the acoustic signal production and thus to compare the courtship behaviour of the different pairs tested. This comparison would not be possible with winged males, some individuals being more acoustically 'active' than others.

All experiments were performed between 09.00 and 13.00 at a temperature of 25 ± 1 °C (measured with a digital thermometer) controlled by a heating-cooling system.

One male was introduced into the chamber with an aspirator via the hole and, after a 2 min period during which the male habituated to its new environment, one female was introduced into the chamber and the experimental session began. Courtship behaviours of the pair were observed for 30 min.

(h) Observation of the courtship: parameters measured

To quantify the courtship success of the male the courtship duration was calculated for all pairs, as the copulation latency minus the courtship latency. The period of observation was limited to 30 min. When a male and a female did not mate within this period their copulation latency was scored as equal to the

maximum time (30 min) and their courtship duration was assigned as a censored observation (Hald 1949). Thus this information is not eliminated but kept as the value of 30 min minus courtship latency. The proportion of matings for each experimental condition was also scored.

(i) Statistical analysis

The data were tested for normality using Lilliefors and Shapiro-Wilk tests (Lilliefors 1967; Shapiro *et al.* 1968). As the data were usually not normally distributed non-parametric analyses were performed. The amounts of cuticular hydrocarbons and the courtship song parameters produced by nhs-*tra* and hs-*tra* males were compared using Mann-Whitney *U*-tests. The distributions of the courtship durations were compared using a survival analysis approach. For each experimental condition, a survival function can be defined as the probability that an individual courtship duration T is longer than a given time t :

$$S(t) = \Pr(T > t).$$

Survival curves $S(t)$ (cumulative proportion of unmated pairs against duration), were plotted using the Kaplan & Meier (1958) product limit estimator. The various survivorship distributions in a given experiment were compared with a Gehan's Wilcoxon test. Briefly, the procedure is as follows: a score was attributed to each duration using Mantel's procedures (Mantel 1967) and then in each experimental situation, a χ^2 value was calculated on the basis of the sums of this score. The number of copulations in each experimental condition were compared using Yate's corrected χ^2 (Sokal & Rohlf 1981). All statistic computations were carried out with STATISTICA V5 software.

3. RESULTS

(a) Cuticular hydrocarbons profile of nhs-*tra*, hs-*tra* and hs-*lacZ* males

The cuticular hydrocarbons produced by almost all the males used in the playback experiment were analysed (54 out of 62 nhs-*tra* and 57 out of 61 hs-*tra* males; see figure 1). Also, in order to disentangle the effects of the gene under hsp control from the effects of the heat shock itself, cuticular hydrocarbons produced by 10 hs-*lacZ* were analysed (see figure 1). The overexpression of UAS-*tra* after a heat shock at early imaginal life reduced the total

Table 1. Parameters (mean \pm s.e.) of pulse and sine songs produced by *hs-tra* and *nhs-tra* males. (The numbers of values measured are in brackets.)

		<i>nhs-tra</i>	<i>hs-tra</i>	p^a
pulse song	IPI (ms)	35.4 \pm 0.2 (1906)	36.5 \pm 0.2 (2587)	< 0.0001
	BD (ms)	227.1 \pm 14.3 (297)	251.5 \pm 14.3 (375)	0.45
	NPB	7.4 \pm 0.4 (297)	7.8 \pm 0.4 (375)	0.63
	%PS	3.5 \pm 0.7 (10)	3.4 \pm 0.4 (10)	0.76
sine song	BSSD (ms)	510.8 \pm 40.9 (132)	418.5 \pm 21.6 (210)	0.28
	%SS	3.8 \pm 0.8 (9 ^b)	3.9 \pm 0.6 (9 ^b)	0.82

^a Mann–Whitney *U*-test.^b The sine song produced by one *nhs-tra* male and one *hs-tra* male was not analysed for technical reasons.

amount of all cuticular hydrocarbons by almost 70%. The mean of the total amount of hydrocarbons detected is of 1597 \pm 41 ng for *nhs-tra* males and of 567 \pm 57 ng for *hs-tra* males (Mann–Whitney: $Z = 8.43$; $p < 0.0001$). This reduction is not due to the effects of the heat shock itself, as control *hs-lacZ* males do not show any differences compared with *nhs-tra* males for the total mean amount of hydrocarbons (1536 \pm 88 ng, Mann–Whitney: $Z = 0.35$; $p = 0.725$). In addition the cuticular profile of such *hs-lacZ* males is not significantly different from the one of wild-type CS males (Sureau 1998).

Interestingly, the cuticular substances were non-randomly reduced in concentration, certain hydrocarbons being selectively lowered in *hs-tra* males compared with *nhs-tra* ones. Thus, the *hs-tra* males produced very few compounds with a known or suspected pheromonal role (5-T, 7-T, 7-P; Ferveur & Sureau 1996) compared with *nhs-tra* males. The typically male compounds of *D. melanogaster* 7-T and 7-P were drastically reduced (25 and 106 ng, respectively per male on average, that is 6 and 20% compared with *nhs-tra* individuals) and 5-T was almost undetectable (see caption of figure 1 for details of the abbreviations).

(b) Courtship song of *nhs-tra* and *hs-tra* males

The courtship songs produced by 10 males of each experimental type were recorded. All the acoustic signals (pulse and sine songs) were analysed during the whole courtship, between the start of courtship until copulation. Values of acoustic parameters of both pulse and sine songs are summarized in table 1.

It appeared that *nhs-tra* and *hs-tra* males produced the same courtship song, as no statistical difference was found between these males for any parameter. Even though there was a small difference between the IPIs produced by the two types of males, such mean values (table 1) are included in the range reported for the species *D. melanogaster* (e.g. Ewing & Bennet-Clark 1968; Ritchie & Kyriacou 1994).

(c) Mating success of *nhs-tra* and *hs-tra* winged males

Thirty-five males were tested for each experimental condition. At the end of the 30 min observation period, the proportions of matings for *nhs-tra* and *hs-tra* winged males were not significantly different (33 of the 35 pairs mated with *nhs-tra*, 29 of the 35 pairs mated with *hs-tra*). Nevertheless, survival analysis of the proportion of unmated

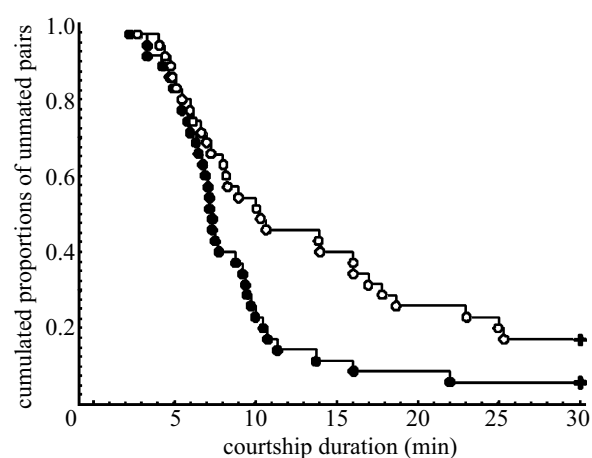


Figure 2. Comparison between survival curves of the unmated state for winged *nhs-tra* (black circles) and *hs-tra* (white circles) males ($n = 35$ in each experimental group). Plus signs correspond to censored data. The distributions are significantly different ($W = 2.016$ 15, $p < 0.05$).

pairs in each group revealed a significant difference (Wilcoxon–Gehan test: $W = 2.016$, $p < 0.05$). As figure 2 shows, the durations of the unmated state were shorter for the males with a normal cuticular profile (*nhs-tra*). It was noticed that the two survival curves followed the same tendency at the beginning and then were clearly separated from 7 to 30 min.

(d) Relative importance of chemical and acoustic signals in mating success

In this experiment (table 2), two factors were tested: the absence or the presence of song, and the abundance or strong reduction of male cuticular hydrocarbons. The lack of both stimuli, acoustic and olfactory, had drastic effects: no copulation was observed during the 30 min observation period. There was a major effect of the song playback on the proportion of matings: among 61 pairs tested in the wingless males plus song situation, 30 (49%) mated within the 30 min period. But among 61 pairs tested in the wingless males plus silence situation only 3 of the 61 (5%) pairs mated ($p < 0.001$). There was also a significant, but less marked effect of the presence of pheromones on the males cuticle: 22 of the 61 pairs (35%) tested with *nhs-tra* males versus 11 of the 61 pairs (18%) with *hs-tra* males succeeded ($p < 0.05$).

Table 2. Proportions of matings in each experimental condition.

percentages (proportions) of matings	winged males		wingless males			
	nhs- <i>tra</i>	hs- <i>tra</i>	nhs- <i>tra</i>		hs- <i>tra</i>	
			plus song	plus silence	plus song	plus silence
	94% (33/35)	83% (29/35)	61% (19/31)	10% (3/30)	37% (11/30)	0% (0/31)

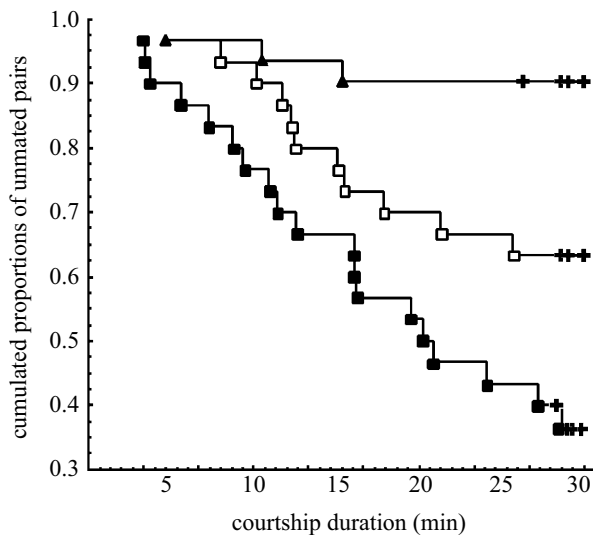


Figure 3. Comparison between survival curves of the state unmated for wingless nhs-*tra* males plus song played back (black squares), wingless nhs-*tra* males plus silence (black triangles) and hs-*tra* males plus song played back (white squares). Plus signs correspond to censored data. The distributions are significantly different ($X^2_2 = 16.55$, $p < 0.001$).

As shown in figure 3, the distributions of the cumulated plots of pairs remaining unmated versus time were significantly different between the three different experimental conditions ($X^2_2 = 16.55$, $p < 0.001$). No copulation occurred in the hs-*tra* wingless males plus silence condition, so this treatment was not included in the statistical comparison. As shown by pairwise comparisons, males with a normal cuticular profile (nhs-*tra*) mated significantly more often and on average after a shorter courtship duration than males with a reduced cuticular profile (hs-*tra*) but with song played back ($W = 1.96$; $p < 0.05$), and than males with a normal cuticular profile but in the absence of song ($W = 4.02$; $p < 0.0001$). There was also a significant difference between the distribution of unmated pairs obtained when the males were hs-*tra* plus playback compared with when males were nhs-*tra* plus silence ($W = 2.32$; $p < 0.05$). At the end of the observation period, significantly more males had mated in the former case than in the latter (see table 2: 37% versus 10%, $p < 0.03$).

4. DISCUSSION

In *D. melanogaster*, as in other *Drosophila* species, information is exchanged during courtship between the two partners through several sensory channels, involving

signals of visual, tactile, chemical and auditory nature (Robertson 1983; Jallon 1984; Tompkins 1984). Our experiments showed that if the male was unable to transmit either chemical or acoustic signals, no matings occurred. So chemical and acoustic cues provide indispensable parameters for the acceptance of males by females, and the simultaneous presence of both factors leads to higher levels of matings (winged nhs-*tra* males and wingless nhs-*tra* males with song played back). In the past, some visual signals such as the movement of circling (Spieth 1974) or the red colour of eyes (Willmund & Ewing 1982) were inferred to have a stimulatory effect on the courtship. According to our experiments, the role of these visual signals, as well as potential tactile signals, seems totally insufficient for stimulating the female to copulate. However, the fact that males do not copulate when they emit neither acoustic nor chemical signals does not mean that visual and tactile signals are useless in the courtship success. Thus, it cannot be excluded that the lack of wings of males in our experiments could be visually detected by the females and therefore could impair the courtship behaviour.

Winged nhs-*tra* males seemed more successful (higher courtship proportions of matings) than wingless nhs-*tra* males with song played back. In the same way, some winged hs-*tra* males seem to mate as rapidly as nhs-*tra* males during the first 7 min (figure 2) compared with mute hs-*tra* males with song played back. Such a difference could be due to at least three reasons. First, as demonstrated by Bennet-Clark & Ewing (1967), when a male vibrates his wings the air movements can act as a stimulus. The wingless males used in our experiments cannot produce air currents and the playback provides only the phasic component of the acoustic stimuli. Second, the playback of signals and the behaviour of the wingless male, which despite the lack of his wings performs a vigorous courtship (including tapping, licking and attempted copulation), could interfere with each other. Hence, the female could be sometimes hampered and confounded by hearing acoustic signals that do not correspond to the behaviour of the male. A third explanation is that wing vibrations could also play a part in the dispersion of males' cuticular hydrocarbons and thus play the role of a mechanical transmitter of the chemical signals together with transmitting an acoustic message.

We have shown that singing males (winged or wingless plus song played back) with a modified hydrocarbon profile were less successful in their courtship than singing males with a normal one. Previous studies demonstrated that chemical cues produced on the cuticle of the female *D. melanogaster* stimulated male courtship behaviour

(Tompkins *et al.* 1980; Venard & Jallon 1980; Antony & Jallon 1982; Ferveur & Sureau 1996). Using *hs-tra* males we have been able to experimentally assess the role of male cuticular hydrocarbons. The hydrocarbon analysis revealed a strong decline in the amount of 7-T, 7-P and a quasi disappearance of the 5-T in *hs-tra* males compared with *nhs-tra* ones. The remaining levels of the 7-T and the 7-P were well outside the natural range observed in wild-type males (Sureau & Ferveur 1999; Rouault *et al.* 2001). The 7-T being the most abundant compound of CS males with a significant volatility was proposed to have a stimulatory role for females (Jallon 1984). This hypothesis was supported by experiments by Scott (1994), although this author did not measure any dose dependence, and was contested by Cobb & Ferveur (1996). The 7-P on the male cuticle could act on the female receptivity but this compound is essentially known for its stimulatory effect on the behaviour of the male (Jallon 1984; Antony *et al.* 1985; Ferveur & Sureau 1996). The semi-volatile 5-T could also be involved in female stimulation, but as our study does not allow us to separate the effects of the above-cited compounds further experiments are needed on this point. In addition, little variation between *hs-tra* and *nhs-tra* males are observed for other compounds, not yet identified for their pheromonal action. We cannot exclude that these compounds could also play a role in the courtship, as it has been demonstrated in experiments with *hs-tra* females (Savarit *et al.* 1999). Moreover, our data clearly show that males with normal levels of cuticular hydrocarbons mate more quickly (see figures 2 and 3), supporting the role of these cuticular compounds to stimulate female acceptance of females.

Nevertheless, we have shown that males with a modified hydrocarbon profile but emitting a normal courtship song (winged *hs-tra*), or with played-back acoustic signals (wingless *hs-tra* plus playback), performed a successful courtship that ended in copulation (83 and 37% respectively). Conversely, when chemical signals were produced alone (wingless *nhs-tra* males plus silence), the proportion of matings observed is very low (only 10%), significantly lower than the proportion of matings obtained if the acoustic signals alone were produced (37%). So, with respect to the relative importance of the two categories of signals but without estimating their quantitative part, it seems that acoustic signals produced by males are of prime importance in stimulating the receptivity of females. In a previous experiment, Markow (1987) showed that deaf mutant female *D. melanogaster* were more handicapped than anosmic mutant females. Thus, the results obtained by experimentally controlling either the reception (as in the Markow study; see also Eberl *et al.* 1997) or the production (as in this study) of signals yield the same results. On the contrary, using mutant females for chemoreception Tompkins *et al.* (1982) and Gailey *et al.* (1986) showed that female mating receptivity was impinged on. However, the nature of the chemoreception defect has not been clearly related to cuticular pheromones. Gailey *et al.* (1986) underlined that the male's odours stimulated the female receptivity only when coupled to singing, but according to these authors, chemical signals were the major stimuli. Nevertheless, in their experiment, the song output was not strictly standardized as they used different individuals producing a

non-controlled amount of song, and as the 1 day old males used could produce non species-specific IPI (Moulin *et al.* 2001).

To conclude, it is impossible to separate out the two sensory modalities. Thus, in our experiments, the effect of the heat-shock treatment on genetically modified males is to halve copulation levels when song is present and to completely abolish them when song is absent. Similarly the effect of removing the wings (but with song still present) is to reduce copulation levels by around one-third when chemical signals are present and by around one-half when chemical signals are strongly modified. Therefore acoustic and chemical signals may act in a synergistic way and not separately.

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As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.